Serial Number: 09/458,779

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Title: SEQUENCE AND METHOD FOR INCREASING PROTEIN EXPRESSION IN CELLULAR EXPRESSION SYSTEMS

(Thrice Amended) [The] A polynucleotide [of claim 3,] comprising SEQ ID NO:1 or a sequence encoding SEQ ID NO:2.

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12. (Four Times Amended) The polynucleotide of claim [3]11, wherein the yeast cell is a Saccharomyces cerevisiae, Schizosaccharomyces pombe, Yarrowia lipolytica, Pichia pastoris, Hansenula polymorpha, or Kluyveromyces lactis.

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- 14. (Thrice amended) A polynucleotide expression vector comprising a polynucleotide encoding a [functional] Vff2p comprising SEQ ID NO:2 or conservative variations thereof[, wherein the Vff2p has greater than 36% sequence identity with SEQ ID NO:2, and wherein the Vff2p increases yeast cell growth or protein secretion].
- 15. (Thrice Amended) [The]An expression vector [of claim 14,] comprising SEQ ID NO:1, or a sequence encoding SEQ ID NO:2.
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- 25. (Twice Amended) A recombinant host cell comprising a yeast cell genetically altered to express a protein encoded by a polynucleotide sequence encoding a functional Vff2p, wherein the Vff2p comprises [has greater than 36% sequence identity with] SEQ ID NO:2 or conservative variations thereof[, and wherein the Vff2p increases yeast cell growth or protein secretion].
  - 26. (Thrice Amended) [The]A host cell [of claim 25,] comprising SEQ ID NO:1, or a sequence encoding SEQ ID NO:2.



31. (Four Times Amended) A method for increasing cell growth of a yeast host cell, comprising introducing a polynucleotide sequence encoding Vff2p into the cell and culturing the cell, wherein the Vff2p comprises [has greater than 36% sequence identity to] SEQ ID NO:2 or conservative variations thereof.

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- 33. (Four Times Amended) A method for increasing protein secretion from a yeast host cell, comprising introducing a polynucleotide sequence encoding Vff2p into the cell and culturing the cell, wherein the Vff2p comprises [has greater than 36% sequence identity to] SEQ ID NO:2 or conservative variations thereof.
- 36. (Four Times Amended) An isolated [functional] vesicular fusion factor 2 protein comprising SEQ ID NO:2 or conservative variations thereof[, or a Vff2p with greater than 36% sequence identity to SEQ ID NO:2, and wherein the Vff2p increases yeast cell growth or protein secretion].
- 37. (Four Times Amended) A method of selecting for a yeast secretory mutant cell containing a polynucleotide sequence encoding a Vff2p operably linked to a promoter, wherein the Vff2p comprises SEQ ID NO:2 or conservative variations thereof, [or a Vff2p greater than 36% identity to SEQ ID NO:2,] the method comprising growing the yeast secretory mutant cell at a restrictive temperature of about 32-37°C, wherein the restrictive temperature selectively favors mutant cell growth.
- 46. (Amended) The method of claim 31, 33 or 37, wherein the yeast cell is a Saccharomyces cerevisiae, Schizosaccharomyces pombe, Yarrowia lipolytica, Pichia pastoris, Hansenula polymorpha, or Kluyveromyces lactis cell.

Please add new claims 47-52.

47. (New) An isolated polynucleotide comprising a sequence encoding a vesicular fusion factor 2 protein comprising SEQ ID NO:2, wherein the vesicular fusion factor 2 protein increases *Saccharomyces cerevisiae* cell growth or protein expression.

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- 48. (New) An isolated polynucleotide comprising SEQ ID NO:1 encoding a vesicular fusion factor 2 protein that increases *Saccharomyces cerevisiae* cell growth or protein expression.
- 49. (New) A polynucleotide expression vector comprising a polynucleotide encoding a vesicular fusion factor 2 protein comprising SEQ ID NO:2, wherein the vesicular fusion factor 2 protein increases *Saccharomyces cerevisiae* cell growth or protein expression.
- 50. (New) A recombinant host cell comprising a *Saccharomyces cerevisiae* cell genetically altered to express a protein encoded by a polynucleotide sequence encoding a vesicular fusion factor 2 protein comprising SEQ ID NO:2.
- 51. (New) A method for increasing cell growth of a *Saccharomyces cerevisiae* host cell, comprising introducing a polynucleotide sequence encoding a vesicular fusion factor 2 protein comprising SEQ ID NO:2 into the cell and culturing the cell.
- 52. (New) A method for increasing protein secretion from a yeast host cell, comprising introducing a polynucleotide sequence encoding a vesicular fusion factor 2 protein comprising SEQ ID NO:2 into the cell and culturing the cell.

## **REMARKS**

Applicant has carefully reviewed and considered the Office Action mailed on March 26, 2002, and the references cited therewith.

Claims 3, 4, 12, 14, 15, 25, 26, 31, 33, 36, 37 and 46 are amended, claims 43-45 are canceled without prejudice and new claims 47-52 are added; as a result, claims 3-12, 14-23, 25-27, 29-34, 36-42, 46 and 47-52 are now pending in this application. Support for newly added claim 47-52 can be found throughout the specification and claims. For example, support for claim 47-52 can be found in claims 3, 4, 14, 25, 31 and 33. Applicant submits that claims 47-52 do not constitute new matter.